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<b>(54) Title:</b> FRESH PRODUCE WASH FOR INCREASING SHELF LIFE  <b>(57) Abstract</b>  A composition is disclosed for increasing the shelf life of fruit, vegetable and animal produce. The composition is also suitable for removing surface contaminants from fruit, vegetable and animal produce. The composition includes: (a) one or more surfactant(s), (b) one or more anti-microbial, fungicidal and/or fungistat agent(s), (c) one or more buffering agent(s) and/or sequestering agent(s), (d) one or more anti-browning agent, and (e) one or more stabiliser(s) and/or processing additive(s). The composition is applied to the produce and optionally, the produce is subsequently rinsed with water.		

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## FRESH PRODUCE WASH FOR INCREASING SHELF LIFE

### Technical Field

The present invention relates to a wash composition for increasing the shelf life of fruit, vegetable and/or animal produce.

### Background Art

Polyphenyl oxidase (PPO) is found in all fruits and vegetables. The activity of PPO is high in foods that are particularly sensitive to oxidative browning, such as potatoes, apples, mushrooms, bananas, peaches, fruit juices and wines. Browning is exacerbated by tissue damage caused by cutting, peeling, comminuting, pureeing, pitting, pulping or freezing. In uncut or undamaged fruits and vegetables, the phenolic substrates are separated from PPO by compartmentalisation, and browning does not occur.

The oxidation of mono- and diphenols to o-quinones, catalysed by PPO, is followed by spontaneous polymerisation or the reaction of o-quinones with amino acids or proteins to form brown pigments (melanins). Since browning is an oxidative reaction, it can be retarded by the elimination of oxygen from the cut surface of the fruit or vegetable. However, this is not always feasible and browning will occur rapidly when oxygen is reintroduced. Thus, a more feasible and practical approach for the prevention of browning is the use of anti-browning agents. Anti-browning agents are compounds that act primarily on either the enzyme or the intermediates of pigment formation. Their use in the food industry is constrained by issues such as toxicity, cost and effects on taste, texture and colour.

The most widespread approach in the food industry for the control of browning is the use of sulphating agents. Sulfites, are potent, non-specific reducing agents and although they are effective at inhibiting both the enzymatic and non enzymatic browning reactions, there is growing concern over their use. Adverse health effects associated with sulfite usage and increased and regulatory scrutiny have stimulated the search for practical, functional alternatives to sulfating agents.

The growth of the vast majority of micro-organisms on fruits and vegetables can be suppressed by storage at refrigeration temperatures. Crops such as apples, grapes and carrots will tolerate temperatures near freezing. Other crops, for example cucumbers, peppers, summer squash, sweet potatoes and mature green tomatoes, will undergo chill injury if stored below about 12°C for more than a few days.

Storage of sound fruits and vegetables under an optimum set of temperature, relative humidity and atmospheric gas composition conditions will result in maximum preservation of sensory qualities. However, such

conditions do not always result in maximum control of growth of micro-organisms naturally present on produce. This method is also expensive and distribution made more difficult.

Flume and spray washing of raw fruits and vegetables can be successfully used to reduce surface populations of micro-organisms. Not all types of produce are amenable to the physical stresses associated with such washing treatments, however, and must therefore be disinfected by subjecting the produce to other treatments. Sterilisation by repeated washing, even with sterile water, cannot be achieved because viable micro-organisms within tissues of produce remain in place.

Chlorine, in an aqueous solution, exhibits rapid microbiocidal action. Lethality has been attributed to chlorine combining with cell membrane proteins to form N-chloro compounds which in turn interfere with cell metabolism. Inhibition of enzymes sensitive to oxidation by chlorine also appears to be involved in the inactivation of micro-organisms. But failure to maintain adequate chlorine in wash water can actually lead to increased microbial populations on produce. In a study designed to determine micro biological changes in fresh market tomatoes during packing operations, it has been observed that total plate counts and populations of enterobacteriaceae increased on tomatoes washed in water. Additionally, the possibility of flavour and depth of colour changes can and do occur.

Ozone ( $O_3$ ) has been investigated as an agent for decontaminating bioclean rooms in hospitals and other facilities used to treat patients with diseases with a great risk of infection. Although not as effective as formaldehyde, ozone is convenient to use and can be rapidly removed from the air after treatment. For these reasons, ozone has some potential for use as a disinfectant for air and surfaces in contained areas in which produce may be stored or processed.

The use of ozone to decontaminate various types of foods has been investigated. Preservation of fish, reduction of aflatoxin in peanuts and cottonseed meals and reduction of microbial populations on poultry, bacon, beef, butter, cheese, eggs, mushrooms, potatoes and fruits using gaseous ozone have been studied. Ozone, at parts per million (ppm) concentrations, is lethal to a wide variety of micro-organisms, including viruses, bacteria, fungi and amoebae. High relative humidity generally favours microbiocidal activity.

The lethal effect of ozone is a consequence of its strong oxidising power. Unfortunately, physiological injury of produce can result from exposure to concentrations as low as 1.5 ppm.

The use of gamma irradiation to kill micro-organisms on a wide range of food types has been studied extensively and have been found to be quite effective. Fresh sweet corn when subjected to 1.0 KGY (CO) irradiation had over a 3-log reduction in aerobic mesophilic micro-organisms and over a 1-log reduction in yeasts. Growth of survivors on irradiated corn resumed only after 10-12 days and proceeded at a somewhat slower pace compared to microflora on non irradiated corn.

While the effects of irradiation on moulds normally associated with spoilage of fruits have been demonstrated to be effective, the method is not commercially viable because of the requirement to include the irradiation method on the label. This would not be favourably perceived by the potential purchaser of the produce.

It is well known that sensory quality of produce can be preserved by storage under an atmosphere with modified carbon dioxide, oxygen and nitrogen content. The optimum percentage of these gasses differs depending upon the fruit or vegetable, the temperature of storage and the desired end use of the product. Microbial growth can also be controlled, within limits, by modifying the composition of gas in contact with fruits and vegetables. However, an extension of sensory shelf life may simultaneously increase the probability that high populations of pathogens will be present on produce at point of consumption. For example, the shelf life of asparagus, broccoli and cauliflower stored under modified atmosphere can be extended substantially but with a corresponding increase in the populations of *L. monocytogenes* and *Aeromonas aerogenes*, thereby increasing the public health hazards associated with consumption of these vegetables.

Reducing agents act by causing the chemical reduction of the pigment precursors. This effect is temporary since the reducing agents are oxidised irreversibly in the reaction. The non-specificity of reducing agents can also result in products with off flavours and/or off colours.

An alternative approach to the prevention of browning, involving the irreversible modification of the phenolic substrates has been proposed. Apple juice has been successfully treated in the presence of ascorbic acid with the bacterial enzyme protocatechuate 3,4-dioxygenase, which catalyses the oxidative ring-opening and o-fission of catechols. However, commercial use of the enzyme is not feasible due to the cost.

Anti-microbial compounds either naturally present in fruits and vegetables or formed in response to physical or chemical stresses can contribute to extending shelf life. A large number of naturally occurring compounds are commonly found in fruits and vegetables. Organic acids, whether naturally

present in raw fruits and vegetables or accumulated as a result of fermentation, have been relied upon for years to control microbial spoilage. Some organic acids behave primarily as fungicides or fungistats, while others are more effective at inhibiting bacterial growth. Acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids are the major organic acids naturally occurring in many fruits and vegetables. The mode of action of these acids is attributed to direct pH reduction, depression of internal pH of microbial cells by ionisation of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability. In addition to inhibiting substrate transport, organic acids may also inhibit NADH oxidation, thus eliminating supplies of reducing agents to electron transport systems.

The use of washes and sprays containing organic acids, particularly lactic acid, has been successful in decontaminating beef, lamb, pork and poultry carcasses. Application of organic acid washes to the surface of fruits and vegetables for the purpose of reducing populations of viable micro-organisms also has potential. Since the undissociated portion of the acid molecule is primarily responsible for anti-microbial activity, effectiveness depends largely upon the dissociation constant(s) (pKa) of the acid. Because the pKa of most organic acids is between pH 3 and 5, surface application would be most effective on fruits. However, treatment of vegetables with an organic acid wash followed by washing with potable water to achieve removal of the acid is also a means of partial disinfection.

In addition to organic acids, medium-chain fatty acids, containing 12-18 carbon atoms, also exhibit anti-microbial activity. Generally, fatty acids are most effective against gram positive bacteria and yeasts, although some are inhibitory to moulds as well. Modes of action have been attributed to outer membrane dysfunction, reduction of oxygen uptake, alterations in cell membrane permeability or uncoupling of the electron transport chain of specific proteins responsible for ATP regeneration and nutrient transport into the cell.

Fatty acid esters of sucrose and other polyhydric alcohols are industrially synthesised for use as emulsifiers in food formulations. However, these compounds are also produced naturally in plants and have been demonstrated to have broad-spectrum anti-microbial activity at ppm concentrations. The effectiveness of medium-chain fatty acids and fatty acid esters of polyhydric alcohols as sanitisers for raw fruits and vegetables is limited by their solubility in water.

The anti-microbial activity of extracts of several types of plant parts used as flavouring agents in foods has been recognised for centuries. The

compounds responsible for this activity are often found in the essential oil fraction. Unfortunately, although the inhibitory constituents is in the parts per billion (ppb) range, their distinct aroma and flavour limit their use for disinfecting fruits and vegetables.

A group of plant anti-microbial chemicals are the phytoalexins. These low-molecular-weight compounds are synthesised by tissues in response to microbial infection or stress conditions such as injury or physiological stimuli. Phytoalexins alter the properties of plasma membranes and inhibit electron transport in mitochondria. They are produced by many different plants and plant tissues, including roots, tubers, stems, leaves, flowers and fruits. Legumes are capable of producing several different phytoalexins having broad-spectrum anti-microbial activity. It is known that carrot tissue fluid is lethal to *L. Monocytogenes*. The active compound may be 6-methoxymellein, although confirmation has not been made. Also reported that 6-methoxymellein inhibits growth of both fungi and bacteria.

Natural anti-browning agents are also known. A novel inhibitor isolated from fig extracts analysis showed 4-substituted resorcinol's. 4-Substituted resorcinol's were also screened for their ability to act as PPO inhibitors. Of the 4-substituted resorcinol's, 4-hexylresorcinol has the greatest potential for use in the food industry due to its low  $IC_{50}$  (the concentration necessary to result in 50% inhibition of enzyme activity in a spectrophotometric assay system), positive results from tests in food systems, and its long safe history of human use in non-food applications.

A water-soluble, stable compound, 4-hexylresorcinol is non-toxic, non-mutagenic and non-carcinogenic and is generally recognised as safe (GRAS) for use in the prevention of shrimp melanosis. Results from laboratory studies indicate that 4-hexylresorcinol also inhibits the browning of fresh and dried apple and potato slices, avocado (in guacamole) and liquid systems such as apple and white grape juices.

The 4-substituted resorcinol's have several advantages over sulfites for use in foods: their activity is specific towards PPO; they do not 'bleach' pigments in the way that excess sulfites can; and they are chemically stable.

The present inventors have found a wash composition which increases the shelf life of fruit and/or vegetables without effecting the produce and which is commercially viable. The composition according to the present invention exploits a unique combination of natural anti-microbial compounds and natural anti-browning agents and is non-toxic.

The use of natural active ingredients gives confidence to the user regarding product safety and improved manageability.

### Disclosure of the Invention

In a first aspect, the present invention is directed to a composition for increasing the shelf life of and/or aiding the removal of surface contaminants from fruit and/or vegetables and/or animal produce, the composition comprising:

- (a) one or more surfactant(s),
- (b) one or more anti-microbial, fungicidal and/or fungistat agent(s),
- (c) one or more buffering agents and/or sequestering agent(s).
- (d) one or more anti-browning agent(s), and
- (e) one or more stabilisers and/or processing additive(s).

In a second aspect, the present invention provides a method for increasing the shelf life of and/or aiding the removal of surface contaminants from fruit and/or vegetables and/or animal produce comprising applying the composition according to the first aspect of the invention to the fruit and/or vegetables and/or animal produce in such a manner so as to increase the shelf life of and/or aid removal of surface contaminants from fruit and/or vegetables and/or animal produce. Optionally, the fruit and/or vegetables and/or animal produce is rinsed with water after the application of the composition.

In a preferred embodiment, the composition and method described in the first and second aspects of the invention aid the removal of pesticides from the surface of the fruit and/or vegetables. The composition according to the present invention is capable of providing a wide HLB (Hydrophilic Lipophilic Balance) range giving the final solution the ability to solubilize a number of currently used pesticides and carriers, thereby aiding the surface removal of contamination from fresh fruit and vegetables. Lower HLB values improve oil solubility and higher HLB values improves water solubility.

The composition according to the present invention combines groups with an affinity for markedly polar surfaces ensuring solubilisation in water and also groups with an affinity for non-polar surfaces.

The surfactants can be selected from anionic, cationic, nonionic and amphoteric compounds and mixtures thereof and may include sucrose esters of fatty acids, alkyl polyglycosides, polydimethylsiloxane and coconut oil derived glutamates. Preferably the anionic surfactants are selected from alkyl benzene sulphonic acids and salts, alkyl ether carboxylic acids and salts, alkyl sulphosuccinamates, alkyl sulphosuccinates, alpha olefin sulphonates, aromatic hydrocarbon sulphonic acids and blends, aromatic hydrocarbon sulphonate salts and condensates, fatty alcohol ethoxy sulphates, fatty alcohol sulphates and phosphate esters. Preferably the cationic surfactants are selected from alkyl dimethylamines and quaternary ammonium compounds, such as, alkyl



dimethyl benzyl ammonium chloride. Preferably, the nonionic surfactants are selected from alkyl phenol ethoxylates, amine oxides, castor oil ethoxylates, ethylene glycol esters, ethylene oxide/propylene oxide condensates, fatty acid dialkanolamides, fatty acid ethoxylates, fatty acid monoalkanolamides, fatty acid monoalkanolamide ethoxylates, fatty amine alkoxylates, fatty alcohol ethoxylates and fatty alcohols. Preferably the amphoteric surfactants are selected from alkyl ampho(di)acetates, amido betaines and amine betaines. Preferably, the concentration of surfactant is between 0.1% to 50% w/w. More preferably, the concentration is between 0.05 to 5% w/w.

The composition contains one or more anti-microbial, fungicidal and/or fungistat agents which may be selected from fatty acids and/or the equivalent fatty acid salts, fatty acid esters, organic acids, and phytoalexins. Preferably, the fatty acids contain 12-18 carbon atoms. Preferably, fatty acid esters of sucrose and other polyhydric alcohols are used. The organic acids may be selected from acetic, succinic, citric, tartaric, malic, ascorbic, benzoic acids, niacin and polyhexanamide. Preferably, the organic acids have a pH between 3 and 5. The corresponding calcium, potassium, and sodium salts of the organic and fatty acids may also be used. Preferably, the anti-microbial, fungicidal and/or fungistat agents are naturally occurring and non-toxic in their desired concentration. Grapefruit extract can be used as both an anti-microbial and fungicide. The anti-microbial, fungicidal and/or fungistat agents are preferably present in a concentration between 0.001 to 50% w/w preferably between 0.001 to 5% w/w and more preferably between 0.001 to 2% w/w. These agents act as disinfectants by improving surface decontamination and inhibiting microbial growth.

The composition may include one or more buffering agents. The buffering agents may be selected from water soluble acids and the corresponding salts, inorganic acids with low pKa and/or organic acids. The buffering agents may be selected from ascorbic acid, acetic acid, adipic acid, benzoic acid, boric acid, citric acid, caprylic acid, cystine-beta-thiopropionic acid, dehydroacetic acid, formic acid, fumaric acid, erythorbic acid, glutamic acid, glutaric acid, hydrochloric acid, lactic acid, malic acid, monohalogenacetic acid, monobromoacetic acid, propionic acid, phosphoric acid, peracetic acid, octanoic acid, salicylic acid, succinic acid, sulphuric acid, sulphonc acid, tartaric acid. The buffering agents are preferably in a concentration of between 0.001% to 50% w/w, more preferably between 0.001 to 2% w/w.

The composition may include one or more sequestering agents. Organic or physical sequestering agents may be selected. Chemical sequestering agents

include ethylenediaminetetraacetic acid (EDTA) and salts, sodium citrate and phosphates. Physical sequestering agents include cyclodextrins. Preferably the sequestering agents are in a concentration of between 0.005% to 50% w/w, preferably between 0.005 to 5.0% w/w and more preferably between 0.05 to 5 %w/w.

The composition includes one or more anti-browning agents which may include organic, inorganic and bioactive compounds which inhibit enzyme activity. The anti-browning agents may be selected from resorcinols such as 4-hexyl resorcinol, erythorbic acid, cysteine hydrochloride, aluminium sulphate, ascorbic acid, sodium ascorbate, dextrose. Preferably, the anti-browning agents are naturally occurring and non-toxic in their resulting concentration. The anti-browning agents preferably will be in a concentration of between 0.0001% to 50% and more preferably between 0.0001 to 1% w/w.

The mechanism of inhibition is quite different for each of the categories of enzymatic browning inhibitors. Synergism has been discovered for several blends of anti-browning agents. Combinations of anti-browning agents, result in more effective inhibition of browning than can be obtained by any single agent on its own.

The composition includes one or more stabilisers and/or processing additives. The stabilisers may be selected from aromatic carboxylic acids, aliphatic alcohols, substituted resorcinols, anions, peptides, oxygenases, o-methyltransferases, proteases and water soluble colloids. Preferably, the aliphatic alcohol is selected from amyl alcohol, butyl alcohol, ethyl alcohol, methyl alcohol, propyl alcohol, benzyl alcohol, glycerol, ethylene glycol, propylene glycol and ethers of the higher aliphatic alcohols. The substituted resorcinol is preferably 4-hexyl resorcinol. Preferred anions include hydroxyl, carbonate and phosphate ions which may be obtained from the corresponding sodium salts. The peptides may be based on amino acids selected from phenylalanine, lysine, tryptophan, alanine and glycine. The water soluble colloids may include glucose, cellulose and starch derivatives. The stabilisers and/or processing additives are preferably in a concentration of between 0.1% to 50%. The stabilisers act as solubilisers and/or antioxidants. They help provide a stabler solution containing high solids and prevent changes in form and/or chemical nature.

The composition may optionally include additional antioxidants and preservatives. The antioxidants and preservatives may be selected from ascorbic acid, sodium ascorbate, erythorbic acid, sodium erythorbate, citric acid, sodium citrate, sorbic acid, potassium sorbate, benzoic acid, sodium

benzoate, parabens, niacin, hexamine, biphenyl, 2-hydroxybiphenyl, 2-benzimidazole and cysteine. Preferably, the preservative is bacteriostatic.

The composition may be produced as a powder or liquid concentrate. Preferably the composition is made up using clean, potable cold water (at ambient temperature). The composition may be applied to the fruit and vegetables by dipping the produce into a solution of the composition or by spraying which is normal, but not necessarily, followed by a water rinse. Preferably, the produce is dipped into a solution of the composition. Typically, the dipping time will be between 1 to 4 minutes, although longer dipping times and greater concentrations may be required for bigger pieces of produce.

The composition of the present invention exhibits a wide range of characteristics, including: low surface tension, high wetting, superior dispensing and emulsifying, moderate foaming/low foaming, broad solubility range, low volatility, good temperature stability, compatible with a broad range of system components, ability to concentrate for economy, and low toxicity - environmentally safe.

The following Examples further illustrate the invention.

#### Examples:

The following compositions are effective in increasing the shelf life of fresh fruit and vegetables.

**Table 1 - Composition 1**

Sample Formula:	Function	% w/w
polyglucose	surfactant	0.15
sucrose esters	surfactant	0.2
polyhexanamides	surfactant/anti-microbial	0.2
EDTA	sequesterant	0.2
niacin	anti-microbial	0.005
grapefruit extract	anti-microbial/fungicide	0.05
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.05
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.1
water	diluent	balance

**Table 2 - Composition 2:**

<b>Sample Formula:</b>	<b>Function</b>	<b>% w/w</b>
polyglucose	surfactant	0.15
sucrose esters	surfactant	0.2
polyhexanides	surfactant/anti-microbial	0.2
EDTA	sequesterant	0.2
niacin	anti-microbial	0.005
grapefruit extract	anti-microbial/fungicide	0.05
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.05
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.3
citric acid	Buffer	0.6
sodium chloride	stabiliser	0.1
water	diluent	balance

**Table 3 - Composition 3:**

<b>Sample Formula:</b>	<b>Function</b>	<b>% w/w</b>
polyglucose	surfactant	0.25
sucrose esters	surfactant	0.2
polyhexanides	surfactant/anti-microbial	0.2
EDTA	sequesterant	0.2
niacin	anti-microbial	0.005
grapefruit extract	anti-microbial/fungicide	0.05
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.1
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.01
citric acid	buffer	0.1
Malic acid	buffer	0.2
Potassium Sorbate	antioxidant/preservative	0.5
water	diluent	balance

**Table 4 - Composition 4**

<b>Sample Formula:</b>	<b>Function</b>	<b>% w/w</b>
polyglucose	surfactant	0.15
sucrose esters	surfactant	0.25
polyhexanides	surfactant/anti-microbial	0.2
EDTA	sequesterant	0.2
niacin	anti-microbial	0.001
grapefruit extract	anti-microbial/fungicide	0.05
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.05
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.1
cysteine	antioxidant/preservative	0.01
erythorbic acid	anti-browning/buffer	0.1
lactic acid	buffer	0.1
water	diluent	balance

**Table 5 - Composition 5**

<b>Sample Formula:</b>	<b>Function</b>	<b>% w/w</b>
polyglucose	surfactant	0.15
sucrose esters	surfactant	0.2
polyhexanides	surfactant/anti-microbial	0.1
EDTA (di-sodium)	sequesterant	0.05
niacin	anti-microbial	0.005
grapefruit extract	anti-microbial/fungicide	0.05
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.05
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.1
benzylammonium chloride	surfactant/anti-microbial	0.15
polyethylene glycol	surfactant	0.10
water	diluent	balance

**Table 6 - Composition 6**

Sample Formula:	Function	% w/w
polyglucose	surfactant	0.15
sucrose esters	surfactant	0.2
polyhexanides	surfactant/anti-microbial	0.2
EDTA	sequesterant	0.2
niacin	anti-microbial	0.005
grapefruit extract	anti-microbial/fungicide	0.01
sodium benzoate	preservative	0.01
sodium citrate	buffer	0.01
substituted resorcinol	anti-browning/stabiliser	0.001
ascorbic acid	anti-browning/buffer	0.1
potassium sorbate	antioxidant/preservative	0.15
water	diluent	balance

The final composition can be in liquid or solid concentrate to be diluted by the user as required. The required diluted solution is made up using clean, potable cold water at ambient temperature. The liquid composition can be sprayed onto the produce or preferably the produce can be dipped in the liquid composition. Typically dipping time varies from 1 to 4 minutes. Some examples of the required dipping times are shown in Table 7

**Table 7 - Dipping times for produce in Composition 1:**

Produce	Dipping time	Result
Potatoes	120-240 seconds (longer times and higher concentrations are required on bigger cuts)	inhibits browning and slows the onset of bruising. May also be effective in conjunction with low SO <sub>2</sub> levels. Shelf life of up to 4 days can be achieved.
Apples: Bramley (diced) Granny Smith (sliced)	120-180 seconds  120-240 seconds	inhibits browning.
Celery	120-180 seconds	prevents browning and callus formation

Peppers	120 seconds	prevents silvering and dehydration of cut surfaces.
Broad beans	120-240 seconds	prevents browning of shelled beans for up to 4 days.
Carrots	60 seconds	prevents silvering and dehydration. May also be used in conjunction with 1% citric acid solution.
Aubergines	120-180 seconds	prevents discolouration and colour leaching.

### Evaluation of the Antimicrobial Effect of "Composition 1"

The following experimental evaluation was carried out using a protocol developed by Campden & Chorleywood Food Research Association.

Selected vegetables and salad products were inoculated with a range of vegetable pathogens and left to dry. The products were then washed with the composition according to the present invention according to manufactures instructions and an assay carried out to assess the number of viable bacteria remaining. Comparisons were set up following the same procedure but using chlorinated water (100ppm) and tap water as controls. In order to establish the natural reduction in bacterial numbers over time an untreated contamination sample was also examined.

### Experimental Detail

Three commonly found vegetable pathogens were used in the experiment namely, *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*. These were grown in broth culture overnight and used to inoculate the surface of the vegetables to a level of approximately  $10^6$  organisms per gram of vegetable. The three vegetables used in this series of experiments were Iceberg lettuce, watercress and radish.

Four samples of each vegetable (24 radishes) were infected and left to air dry for two hours before examination. The lettuce was separated into individual leaves and dried initially in a vegetable "centrifuge" for two minutes to remove excess moisture before leaving to air dry.

After two hours one of each of the vegetables (six radishes) were washed in "Composition 1", chlorinated water and tap water, the remainder being left

untreated. These were then analysed in triplicate for the presence of each of the bacteria used to infect the vegetables. The unused portions of vegetables were then placed in a refrigerator and stored at 5°C for two days. After 48 hours storage at this temperature the samples were again examined for the present of the three bacteria. A schematic representation of the experimental protocol is shown in Table 8.

**Table 8:**

Untreated	100ppm Chlorine wash	Tap water wash	"Composition 1" (0.5%)
Air dry for 2 hours	Air dry for 2 hours	Air dry for 2 hours	Air dry for 2 hours
Analyse 3 samples	Analyse 3 samples	Analyse 3 samples	Analyse 3 samples
Store @ 5°C for 48 hours	Store @ 5°C for 48 hours	Store @ 5°C for 48 hours	Store @ 5°C for 48 hours
Analyse 3 samples	Analyse 3 samples	Analyse 3 samples	Analyse 3 samples

The results obtained from this experimental protocol are tabulated below in Tables 9 to 11. All figure quoted are organisms per gram of sample.

**Table 9 - Iceberg lettuce:**

	Salmonella Typhimurium	Listeria monocytogenes	Escherichia coli
Chlorine wash 0 hrs	350	480	450
48 hrs	20	40	40
Tap Water 0 hrs	4800	3700	5800
48 hrs	3100	2100	3000
"Composition 1" 0 hrs	210	350	390
48 hrs	<10	30	<10
Control 0 hrs	$9.8 \times 10^4$	$8.1 \times 10^4$	$1.16 \times 10^5$
48 hrs	$8.2 \times 10^4$	$7.3 \times 10^4$	$8.1 \times 10^4$



**Table 10 - Watercress:**

	Salmonella Typhimurium	Listeria monocytogenes	Escherichia coli
Chlorine wash 0 hrs	1020	680	860
48 hrs	90	40	40
Tap Water 0 hrs	7800	5600	14100
48 hrs	5100	3600	9100
"Composition 1" 0 hrs	880	550	580
48 hrs	30	50	30
Control 0 hrs	$1.32 \times 10^5$	$9.3 \times 10^4$	$2.14 \times 10^5$
48 hrs	$1.68 \times 10^5$	$1.04 \times 10^5$	$2.06 \times 10^5$

**Table 11 - Radishes:**

	Salmonella Typhimurium	Listeria monocytogenes	Escherichia coli
Chlorine wash 0 hrs	320	190	210
48 hrs	<10	<10	<10
Tap Water 0 hrs	5800	4800	6300
48 hrs	3100	2000	2200
"Composition 1" 0 hrs	260	200	180
48 hrs	<10	<10	<10
Control 0 hrs	$2.88 \times 10^5$	$1.54 \times 10^5$	$4.74 \times 10^5$
48 hrs	$1.31 \times 10^5$	$8.32 \times 10^5$	$2.55 \times 10^5$

**Conclusion**

The results show a consistent pattern of reduction of bacterial numbers with a straight cold water wash being quite efficient but still leaving on average some 5% of the initial infection behind after initial wash and 2-3% after 48 hours. By contrast the chlorine wash showed a marked reduction with a retention of only 0.7% on the cress and only 0.1% on the radishes.

"Composition 1" according to the present invention was consistently the better of the two chemical treatments although the difference between this and a chlorine wash was marginal giving retention of 0.6% on the cress and 0.09% on the radishes. Both chemical treatments removed the bacteria completely after

48 hours. "Composition 1" according to the present invention was however much more comfortable to handle and at 0.5% did not give the fumes experienced using chlorine at 100ppm.

**Examination of effectiveness on freshness and quality of vegetables under "normal" household storage conditions.**

Three fresh lettuces were purchased and transferred to the laboratory. One was left untreated ie no wash, the second was washed thoroughly in cold running water and the third washed in "Composition 1" following the manufacturers instructions. Each was then tested for microbiological quality testing for Total Bacterial Count and Total Coliform Count. An observation was also made as to the appearance and quality of each lettuce. The three were then stored in a refrigerator at 4°C for up to 7 days. Testing for microbiological quality and appearance were carried out at 3, 5 and 7 days. The results obtained are detailed below in Table 12 and 13.

Sample A No Treatment

Sample B Cold Water Wash

Sample C "Composition 1"

**Table 12:**

	A		B		C	
	TVC	Colif	TVC	Colif	TVC	Colif
<b>On receipt</b>	$1.95 \times 10^5$	870	$2.09 \times 10^5$	550	$1.72 \times 10^5$	380
<b>After treatment</b>	-	-	$1.62 \times 10^5$	410	$1.16 \times 10^5$	215
<b>Day 3</b>	$8.8 \times 10^7$	510	$4.3 \times 10^7$	290	$4.7 \times 10^5$	60
<b>Day 5</b>	$9.1 \times 10^7$	70	$7.6 \times 10^7$	230	$6.4 \times 10^5$	20
<b>Day 7</b>	$7.3 \times 10^7$	50	$1.2 \times 10^5$	110	$6.0 \times 10^5$	10

Table 13:

Visual Quality	A	B	C
Day 0	All had firm green leaves. no browning or other spoilage.		
Day 3	Still fresh looking. no spoilage.		
Day 5	Very limp but still green	Firm and fresh looking	
Day 7	No firmness, leaves deteriorating, green colour fading	Brown edges to leaves. Starting to go limp	No Spoilage, leaves still firm and whole

Tables 12 and 13 demonstrate the effectiveness of the wash to reduce browning, maintain crispness and extend the useable life of fresh vegetables. It has been demonstrated that use of Composition 1 on fresh fruit and vegetables improves the safety by reducing the surface microbial count, improves the flavour by surface cleaning of contaminants, extends the freshness and extends the safe useable life.

#### **Minimum Inhibitory Concentration:**

Table 14:

Organism	MIC % concentration
Salmonella typhimurium NCTC 12023	0.8
E Coli NCTC 10418	0.8

Table 14 demonstrates the effectiveness of Composition 1 to inhibit microorganism growth. The solution acts as an effective disinfectant/preservative. The results show a similar MIC to phenolic disinfectant and quaternary type formulations normally sold in the market place. In contrast, however, the Composition of the present invention is safe to use in direct food contact.

#### **Synergism - Anti-browning and wilting tests:-**

The effects of various anti-browning materials and combinations, on vegetables (lettuce) under "normal" household storage conditions were examined.

Four fresh lettuces were purchased and transferred to the laboratory. Each were individually washed in sample solution A, B, C and D (see below).

Each were then stored in a refrigerator at 4°C for 7 days. Observation testing was carried out for visual quality and fresh appearance at 3, 5 and 7 days. The results obtained are detailed below:

#### Laboratory Test - Anti-Browning - Wilting

Ingredient	% w/w			
	A	B	C	D
Alkyl polyglycoside	0.15	0.15	0.15	0.15
Sucrose esters	0.2	0.2	0.2	0.2
EDTA	0.2	0.2	0.2	0.2
Sodium benzoate	0.01	0.01	0.01	0.01
Sodium citrate	0.05	0.05	0.05	0.05
Ascorbic acid	0.1	-	-	0.1
Erythorbic acid	-	0.1	-	0.1
4 Hexylresorcinol	-	-	0.001	0.001
Water	Balance	Balance	Balance	Balance

#### Visual and Sensory Evaluation

	A	B	C	D
Day 0	All firm green leaves. No browning or spoilage			
Day 3	All still fresh. No browning			
Day 5	Limp but green	Firm. fresh	Firm. fresh	Firm. fresh
Day 7	Very limp. fading green to brown	Limp, brown edging	Very limp, green	Firm, fresh

The results demonstrate synergism between blends of anti-browning agents.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

## THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A composition for increasing the shelf life of and/or aiding the removal of surface contaminants from fruit, vegetable or animal produce, the composition including:
  - (a) one or more surfactant(s),
  - (b) one or more anti-microbial, fungicidal and/or fungistat agent(s),
  - (c) one or more buffering agent(s) and/or sequestering agent(s),
  - (d) one or more anti-browning agent, and
  - (e) one or more stabiliser(s) and/or processing additive(s).
2. A composition according to claim 1 wherein the surfactant is selected from anionic, cationic, nonionic and amphoteric compounds and mixtures thereof.
3. A composition according to claim 1 wherein the surfactant is selected from sucrose esters of fatty acids, alkyl polyglycosides, polydimethylcyclonexene and coconut oil derived glutamates and mixtures thereof.
4. A composition according to claim 1 wherein the surfactant is an anionic surfactant selected from alkyl benzene sulphononic acids and salts, alkyl ether carboxylic acids and salts, alkyl sulphosuccinamates, alkyl sulphosuccinates, alpha olefin sulphonates, aromatic hydrocarbon sulphononic acids, aromatic hydrocarbon sulphonate salts and condensates, fatty alcohol ethoxy sulphates, fatty alcohol sulphates, phosphate esters and mixtures thereof.
5. A composition according to claim 1 wherein the surfactant is a cationic surfactant selected from alkyl dimethylamines and quaternary ammonium compounds, preferably, alkyl dimethyl benzyl ammonium chloride.
6. A composition according to claim 1 wherein the surfactant is a nonionic surfactant selected from alkyl phenol ethoxylates, amine oxides, castor oil ethoxylates, ethylene glycol esters, ethylene oxide/propylene oxide condensates, fatty acid dialkanolamides, fatty acid ethoxylates, fatty acid monoalkanolamides, fatty acid monoalkanolamide ethoxylates, fatty amine alkoxyates, fatty alcohol ethoxylates, fatty alcohols and mixtures thereof.
7. A composition according to claim 1 wherein the surfactant is an amphoteric surfactant selected from alkyl ampho(di)acetates, amido betaines, amine betaines and mixtures thereof.

8. A composition according to any one of claims 1 to 6 wherein the surfactant is included in an amount in the range of 0.1 to 50% w/w based on the total weight of the composition, preferably 0.05 to 5% w/w.
9. A composition according to claim 1, wherein the antimicrobial, fungicide and/or fungistat agent is selected from fatty acids, fatty acids salts, fatty acid esters, organic acids and calcium, potassium and sodium salts thereof and phytoalexins.
10. A composition according to claim 9 wherein the fatty acids are C<sub>12-18</sub> fatty acids and the fatty acid salts are calcium, potassium or sodium salts.
11. A composition according to claim 9 wherein the fatty acid esters are esters of polyhydric alcohols, preferably sucrose.
12. A composition according to claim 9 wherein the organic acids are selected from acetic acid, succinic acid, citric acid, tartaric acid, malic acid, ascorbic acid, benzoic acid, niacin, polyhexanamide, and calcium, potassium and sodium salts thereof.
13. A composition according to any one of claims 1 and 9 to 12 wherein the antimicrobial, fungicidal and/or fungistat agents are included in an amount in the range of 0.001 to 50% w/w based on the total weight of the composition, preferably 0.001 to 5% w/w and more preferably 0.001 to 2% w/w.
14. A composition according to claim 1 wherein the buffering agent is selected from ascorbic acid, acetic acid, adipic acid, benzoic acid, boric acid, citric acid, caprylic acid, cystine-beta-thiopropionic acid, dehydroacetic acid, formic acid, fumaric acid, erythorbic acid, glutamic acid, glutaric acid, hydrochloric acid, lactic acid, malic acid, monohalogenacetic acid, monobromoacetic acid, propionic acid, phosphoric acid, peracetic acid, octanoic acid, salicylic acid, succinic acid, sulphuric acid, sulphonic acid, tartaric acid and mixtures thereof.
15. A composition according to any one of claims 1 and 14 wherein the buffering agent is included in an amount in the range of 0.001% to 50% w/w based on the total weight of the composition, preferably 0.001 to 2% w/w.

16. A composition according to claim 1 wherein the sequestering agent is selected from ethylenediaminetetraacetic acid (EDTA) and salts thereof, sodium citrate and phosphate and cyclodextrins.

17. A composition according to claims 1 or 16 wherein the sequestering agent is included in an amount in the range of 0.005% to 50% w/w based on the total weight of the composition, preferably 0.005 to 5.0% w/w and more preferably 0.05 to 5 %w/w.

18. A composition according to claim 1 wherein the anti-browning agent is selected from resorcinol, erythorbic acid, cysteine hydrochloride, aluminium sulphate, ascorbic acid, sodium ascorbate, dextrose and mixtures thereof.

19. A composition according to claim 1 wherein the anti-browning agent is a mixture of 4-hexylresorcinol, erythorbic acid and ascorbic acid.

20. A composition according to any one of claims 1, 18 and 19 wherein the anti-browning agents is included in an amount in the range of 0.0001% to 50% w/w based on the total weight of the composition, preferably 0.0001 to 1% w/w.

21. A composition according to claim 1 wherein the stabilisers are selected from aromatic carboxylic acids, aliphatic alcohols, substituted resorcinols, anions, peptides, oxygenases, o-methyltransferases, proteases, water soluble colloids and mixtures thereof.

22. A composition according to claim 21 wherein the aliphatic alcohols are selected from amyl alcohol, butyl alcohol, ethyl alcohol, methyl alcohol, propyl alcohol, benzyl alcohol, glycerol, ethylene glycol, propylene glycol and ethers of the higher aliphatic alcohols.

23. A composition according to claim 21 wherein the substituted resorcinol is 4-hexyl resorcinol.

24. A composition according to claim 21 wherein the anions are selected from hydroxyl, carbonate and phosphate ions which may be obtained from the corresponding sodium salts.

25. A composition according to claim 21 wherein the peptides are based on amino acids selected from phenylalanine, lysine, tryptophan, alanine and glycine.
26. A composition according to claim 21 wherein the water soluble colloids are selected from glucose, cellulose and starch derivatives.
27. A composition according to any one of claims 1 and 21 to 26 wherein the stabilisers and/or processing additives are included in an amount in the range of 0.1% to 50% w/w based on the total weight of the composition.
28. A method of increasing the shelf life of fruit, vegetable and/or animal produce including the steps of:
- (a) applying to the fruit, vegetable and/or animal produce a composition according to any one of claims 1 to 27; and
  - (b) optionally, rinsing the fruit, vegetable and/or animal produce with water.
29. A method of aiding removal of surface contaminants from fruit, vegetable and/or animal produce including the steps of:
- (a) applying to the fruit, vegetable and/or animal produce a composition according to any one of claims 1 to 27; and
  - (b) optionally rinsing the fruit, vegetable and/or animal produce with water,
30. A method of increasing the shelf life of fruit, vegetable and/or animal produce including the steps of:
- (a) preparing an aqueous solution of a composition according to any one of claims 1 to 27;
  - (b) applying the aqueous solution to fruit, vegetable and/or animal produce;
  - (c) optionally rinsing the fruit, vegetable and/or animal produce with water.
31. Fruit, vegetable and/or animal produce treated by contacting with a composition according to any one claims 1 to 27.



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU 99/00046

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int Cl <sup>6</sup> : A23L 3/34		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) WPAT: A23L 3/34, 3/3463, 3/3481, 3/349, 3/3508, 3/3517, 3/3526, 3/3535, 3/3544, 3/3553; A23B 4/10, 5/06, 7/16. Chemical Abstract (CA) see key words in the electronic database box below.		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Food Science and Technology Abstracts (FSTA) see key words in the electronic database box below		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) See strategy on the extra sheet.		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	R. Ahvenainen, New approaches in improving the shelf life of minimally processed fruit & vegetables, Trends in Food Science & Technology, Vol 7, June 1996 pp 179-187 Whole document	1-31
Y	J. Rice, Organic Acid sprays, Food Processing 55(4), 1994 pp 45, 47-48, 50 Whole document	1-31
X	AU 16317/95 (H. B. FULLER COMPANY) 16 November 1995 Table I page 8	1, 8, 9, 12, 14, 15, 17, 20, 28-31
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 30 March 1999		Date of mailing of the international search report - 7 APR 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer  J.H. CHAN Telephone No.: (02) 6283 2414

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00046

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	WO 95/07616 (MINNESOTA MINING & MANUFACTURING COMPANY) 23 March 1995. Page 3, claim 1	1-31
X, Y	EP 0312519 (MONSANTO COMPANY) 19 April 1989. Page 3 lines 1-35	1-31
X	EP 0245928 (DIVERSEY CORPORATION) 19 November 1987. Whole document 99	1-31
X	EP 0253535 (AGRICULTURAL & FOOD RESEARCH COUNCIL) 20 January 1988. Pages 4-5 esp. page 5 line 33	1-31
X	WO 89/12399 (PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK) 28 December 1989. Page 11 Example 3	1, 2, 14-17, 28-31

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00046

### Continuation of Box B

SS1.	Decontam? Or Sterilis? Or Steriliz? Or Disinfect? Or Sanitis?
SS2	Surfact? Or Emulsif? Or Stabilis? Or Stabiliz?
SS3	Antimicrob? Or Biocid? Or Fungi? Or Bacterioc?
SS4	Antibrown? Or (ascorbic acid) or (citric acid)
CA:	(17/sx or 17/cc) and (SS1 and SS2 and SS3 and SS4)
FSTA	(Hygiene/CT or SS4 or resorcinol) and SS1, and SS3 and (wash? or Dip? or Spray? or Bath?) and (fruit or vegetable or meat)

# **INTERNATIONAL SEARCH REPORT** **Information on patent family members**

International application No.  
PCT/AU 99/00046

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	16137/95	CA	2144021				
WO	95/07616	AU	78684/94	CA	2169559	EP	719089
		US	5460833	US	5490992		
EP	312519	AU	18536/88	CN	1031931	IL	86899
		JP	1039966	PT	87891	US	4770884
		ZA	8804659				
EP	245928	CA	1275245	CA	1275918	DK	1342/87
		US	4715980	US	4776974		
EP	253535	AU	75015/87	DK	3407/87	US	4837037
WO	98/12399	AU	46511/97	US	5749199		
END OF ANNEX							